# Mouse Bone Marrow Monocyte Isolation and Culture Kit

**Cat. No. : P-CA-712**

**Size : 3Tests / 10Tests**

## Background

The Mouse Bone Marrow Monocyte Isolation and Culture Kit is specifically developed for the extraction of primary Mouse Bone Marrow Monocytes. Verified through standardized procedures, each Test of this kit supports the acquisition of one flask of cells (T-25 culture flask), with a cell count exceeding $1\times10^6$ cells. These cells are terminally differentiated and non-proliferative. Following separation. It is recommended to inoculate it directly in the corresponding culture vessel according to the experimental requirements after separation. Through immunofluorescence analysis, the cell purity (CD68 positive rate) has been confirmed to exceed 90%.

## Scope of Application

This product is suitable for extracting Mouse Bone Marrow Monocytes from 20-30-day-old mice of various strains, such as KM and C57. After processes of tissue isolation,and 3-4 days plating, a yield of $>1\times10^6$ cells can be obtained.

**Note**: The intact tibia and femur tissue extracted from 3 mice,typically yield enough cells for one T-25 flask. The exact number of rats required may vary depending on the size and quantity of tibia and femur tissue harvested during this procedure. If the amount of tissue obtained is insufficient, additional experimental mice may be needed to prevent cell quantity deficiency.

## Kit Components

| Name | Size | Appearance | Storage and Expiration Date |
|---|---|---|---|
| Specialized Washing Solution for Mouse Bone Marrow Monocytes | 3Tests (250 mL)<br>10Test (500 mL×2) | Pale Yellow Clear Liquid | 2-8°C, 1 year |
| Specialized Isolation Solution for Mouse Bone Marrow Monocytes | 3Tests (15 mL)<br>10Tests (50 mL) | Colorless Clear Liquid | 2-8°C, 1 year |
| Basic Culture Medium for Mouse Bone Marrow Monocytes | 3Tests (100 mL)<br>10Tests (300 mL) | Red Clear Liquid | 2-8°C, 1 year |
| Supplement for Mouse Bone Marrow Monocytes | 3Tests (10 mL)<br>10Tests (30 mL) | Yellow Clear Liquid | -5~-20°C, 1 year |
| 70 μm Cell Filter | 3Tests (3 pcs)<br>10Tests (10 pcs) | Orange | Room temperature, 3 years |

**Note: All components should be stored according to the temperature indicated on the labels of the reagent tubes. The reagents stored at -5 to -20°C (such as Supplement for Mouse Bone Marrow Monocytes) can be preserved at 4°C for 30 days after thawing. For long-term storage, aliquot them into single-use portions and store at -20℃. Thaw again at 4°C before use to avoid repeated freeze-thaw cycles.**

## Precautions

1. Prior to formal experiments, it is recommended to conduct anatomical simulation training using 1-2 normal mice to familiarize yourself with operational procedures and improve tissue isolation efficiency.

2. Reagent preparation or aliquoting must strictly adhere to aseptic techniques. After dispensing, seal the containers immediately with a sealing film, use them promptly to avoid repeated freeze-thaw cycles or contamination.

## Operational Procedures

**1. Pre-experiment Preparations**

1) Self-supplied Reagents and Consumables: two Eppendorf (EP) tube racks, Phosphate-Buffered Saline (PBS), surgical instruments (At least 3 pairs of ophthalmic scissors, 3 pair of straight forceps, 3 pairs of curved forceps), 2 mL syringe, 6 cm/10 cm culture dishes, T25 culture flasks, dissection board (foam board can substitute) and multiple 2 mL/15 mL/50 mL centrifuge tubes.
2) Reagent Thawing and Rewarming:
   ① Supplement for Mouse Bone Marrow Monocytes: Thaw at 4°C and equilibrate to room temperature.
   ② Specialized Washing Solution forMouse Bone Marrow Monocytes & Basic Culture Medium for Mouse Bone Marrow Monocytes & Specialized Lysis Isolation Solution for Mouse Bone Marrow Monocytes: Equilibrate to room temperature.
3) Preparation of Complete Culture Medium: Add 10 mL of Supplement for Mouse Bone Marrow Monocytes into 100 mL of Basic Culture Medium for Mouse Bone Marrow Monocytes, then mix thoroughly.

   **Note: Storage conditions for complete culture medium: 2-8℃, valid for 3 months. When preparing the complete culture medium, it can be prepared according to the usage amount. Remaining supplement should be aliquoted proportionally and stored at -20℃ to avoid repeated freeze-thaw cycles.**

**2. Dissection Procedures**

1) Animal Euthanasia and Disinfection Protocol Perform euthanasia via pentobarbital sodium overdose injection or cervical dislocation, then immerse the carcass in 75% medical-grade ethanol for 5 minutes for disinfection. After disinfection, transfer the animal to a clean bench for subsequent procedures.
2) Dissection and Tissue Harvesting Steps:
   ① Preparation: Arrange sterilized scissors and forceps in pairs in order of use from left to right on two sterilized EP tube racks(Ophthalmic Scissors 1 and Straight Forceps 1, Ophthalmic Scissors 2 and Curved Forceps 2).

   **Note: The distal third of the instruments should extend beyond the rack to avoid contamination. After each use, return tools to their original positions and make sure they don't touch each other to prevent cross-contact.**

   ② Mouse Fixation: Secure the mouse in a supine position on the clean bench using needles, preparing for tissue harvesting.
   ③ Tissue Harvesting Procedure:
      a. Using Straight Forceps 1 to grasp the instep skin of the hind leg, cut the skin bilaterally from bottom to top with Ophthalmic Scissors 1, and cut up to the abdomen.

      **Note: The entire leg was exposed, while the caput femoris and the calcaneus (ankle bone at the heel) remained uncovered by fur.**

      b. Useing Curved Forceps 2 with the left hand to hold the ankle bone, Use Ophthalmic Scissors 2 to cut the ankle bone with Ophthalmic Scissors 2, lift the ankle bone, use

Ophthalmic Scissors 2 to cut the muscle and skin behind the leg to the joint at the root of the thigh, and cut the joint to get the complete femur and tibia. Transfer the specimen to a culture dish and add 10 mL of Specialized Washing Solution for Mouse Bone Marrow Monocytes. (Figure 1).

**Note: pay attention to keep hair pulled away from the anatomical area. Before sampling, observe the position of tibia and femur to avoid cut off the tibia and femur. If cut off, bone marrow is exposed and bone marrow cavity contacts with muscle. The tissue should be discarded to prevent tissue contamination.**

**3. Tissue Processing and Digestion**

1) Tissue Processing

① Put Straight Forceps 3, Curved Forceps 3 and Ophthalmic Scissors 3 on the EP tube rack within the clean bench, ensuring the distal third of each tool suspended.

② Perform tissue dissection using set of new Ophthalmic Scissors and Curved Forceps. Rinse the tissue once and place it into a new petri dish containing 10 mL of Specialized Washing Solution for Mouse Bone Marrow Monocytes.

③ Bone Tissue Dissection:

a. Use straight forceps 3 to fix the tissue, and the muscle tissue was loosened by bending forceps 3. Pull off large pieces of muscle tissue and avoid thorough cleaning (Figure 2). The bone was placed into a new culture dish, and 10 mL of Specialized Washing Solution for Mouse Bone Marrow Monocytes was added to wash it back and forth.

b. Use straight forceps 3 and curved forceps 3 to grasp the lateral bone of the knee joint , and forcefully pry ait apart in the direction opposite to joint movement (Figure 3). Be careful not to break the bone, and separate the complete femur and tibia (Figure 4). Place the tibia and femur into a new culture dish and add 10 mL of Specialized Washing Solution for Mouse Bone Marrow Monocytes. (Figure 4).

c. Use straight forceps 3 and curved forceps 3 to remove the residual muscle tissue on the surface of femur and tibia (Figure 5), and keeping the bone intact, and leaving the pure femur and tibia (Figure 6). Transfer the tissue to a new culture dish, add 10 mL of Specialized Washing Solution for Mouse Bone Marrow Monocytes, and store for later use.

d. The bone was picked up one by one with bent forceps 3, and the two ends of the bone were cut off with ophthalmic scissors 3 to expose the bone marrow (Figure 7), which was placed in a dry sterile culture dish .

e. Prepare a new culture dish and add 10 mL of Specialized Washing Solution for Mouse Bone Marrow Monocytes. Use the left hand to pick up a bone with a curved forceps, and take a 2 mL syringe with your right hand to draw the Specialized Washing Solution for Mouse Bone Marrow Monocytes from the culture dish (Figure 8). Insert a needle into the thick end of the bone on the petri dish, being careful not to let the bone fall into the dish. Stir the bone marrow with the needle and flush it (Figure 9), continuing until the bone turns white and translucent (Figure 10). Collect the bone marrow fluid in the culture dish (Figure 11) and gently aspirate about 15 times with a 5 mL pipette or a Bacto pipette.

2) Cell Isolation

① Place a 70 μm cell filter on a new 50 mL centrifuge tube. Pre-wash the filter with 1 mL Specialized Washing Solution for Mouse Bone Marrow Monocytes.

Use a 5 mL pipette or a Pasteur pipette to carefully aspirate the bone marrow suspension from the previous step and filter it through a 70 μm cell filter. After filtering, use a clean pipette tip to slowly add 2 mL of Specialized Washing Solution for Mouse Bone Marrow Monocytes over the filter to collect the bone marrow suspension on the filter. Collect the filtrate in a 15 mL centrifuge tube.

**Note: If filtration is impeded, slightly tilt the filter to reduce vacuum seal between the filter and the tube rim.**

② Take a 15 mL centrifuge tube and centrifuge at 1500 rpm for 5 minutes; Discard the supernatant and retain the cell pellet.

**Note: If filtration is impeded, slightly tilt the filter to reduce vacuum sealing against the tube rim.**

**4. Cell Culture and Subculture**

1) Cell Seeding: Take out the culture dish or T25 cell culture flask for the next experiment, and resuspend the cell pellet in the centrifuge tube with 10 mL of Complete Culture Medium of Mouse Bone Marrow Monocytes, then inoculat into the culture dish. The cells were cultured in a incubator at 37°C, 5% $CO_2$.
2) Medium Renewal: Perform the first medium change at Day 3.Under the microscope, if the cell convergence degree is more than 80%, the supernatant can be discarded directly and the fluid can be changed. If most of the cells are not attached to the wall, the fluid can be centrifuged and changed.Followed by subsequent replacements every 2-3 days. Cells typically reach 80-90% confluency within 3-4 days post-seeding.
3) Cell Subculture: It belongs to the terminal differentiated cell group and does not proliferate. Therefore, it is recommended to isolate the cells and inoculate them into the plate as needed for the experiment. Digestion should be avoided as far as possible.
4) Digestion method:
   ① Aspirate the culture medium from the T25 cell culture flask and wash the cells once with PBS. Add 1mL of lidocaine (12 mM) digestion solution to the culture flask, gently rotate the flask to ensure the digestion solution covers the entire bottom of the flask, and incubate at 37°C for 3 minues (not exceeding 5 minues). Under an inverted microscope, observe until the cells have retracted and become round. Then, add 5 mL of complete medium to dilute the digestion solution. Gently mix and disperse the cells using a pipette, aspirate the cell suspension, and centrifuge at 1200rpm for 5 minutes, discarding the supernatant. Resuspend the cells in complete medium, count them, and inoculate them into the appropriate experimental vessels. Once the cells are fully adhered to the walls, culture and observe them for use in the experiment. Afterward, replace the culture medium with fresh complete medium according to the specified frequency.
   ② If the cells fail to digest, replace the digestive fluid with 0.25% trypsin and repeat the above digestion procedure. If the cells still fail to digest, add 3 mL complete medium to terminate the digestion and use a sterile cell scraper to directly scrape the cells (this method is not recommended because the cells will die due to mechanical damage).

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Low yield/low viability | The bone marrow was not washed clean | The bone marrow should be washed out as much as possible until the bone cavity is white to the naked eye |
| | hortage of tissue sampling amount | If more tissue is discarded due to bone cutting or breaking, the number of mice can be increased appropriately |
| The cell adheres slowly | Improper preparation of culture medium | Prepare complete culture medium with accurate ratios and avoid repeated freeze-thaw cycles |
| | | Use the complete culture medium within its validity period and avoid preparing it for more than three months |
| | The age of the mouse is inappropriate | Use mouse aged 20-30 days postnatally .If cells do not adhere to the wall due to different days of age, centrifuge and change the culture for several days |
| Low cell purity | The muscle tissue was not cleaned up and fell into the bone marrow lavage fluid | Ensure the muscle tissue are cleaned out |
| | The bone is broken and the bone marrow fluid is exposed in the muscle tissue, and it's still used | In this case, the bone tissue must be discarded, and the number of cells can be increased by using more mice |

## Anatomy Images for Reference

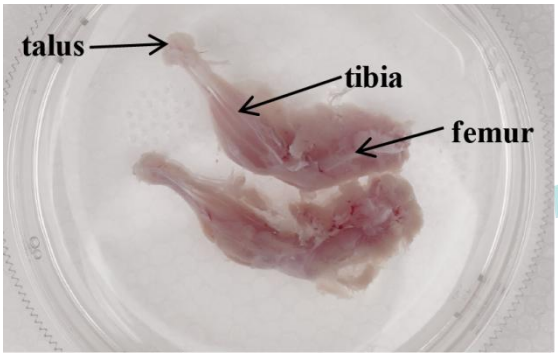


**Figure 1.Get the complete femur and tibia.**

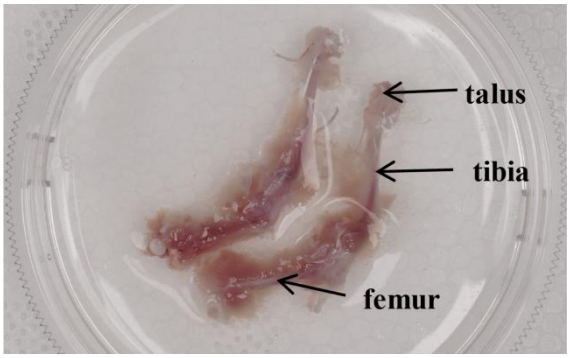


**Figure 2. Pull off large pieces of muscle tissue and avoid thorough cleaningl.**

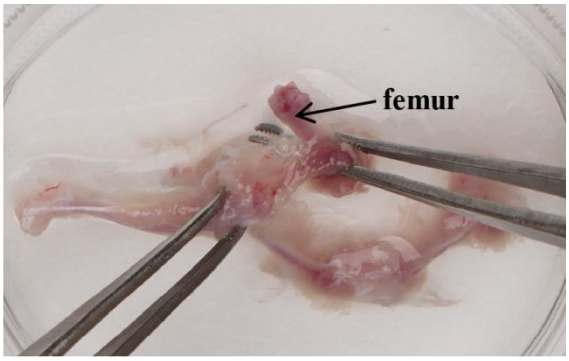


**Figure 3a. Pry it apart in the direction opposite to joint movement.**

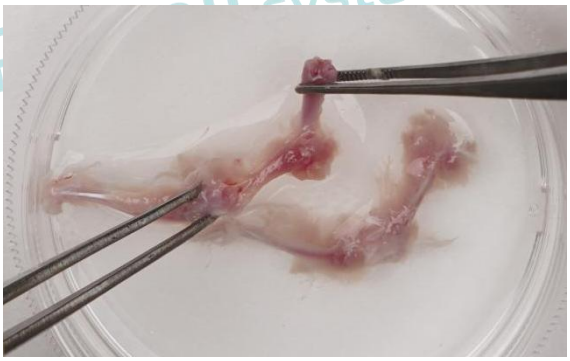

**Figure 3b. Pry it apart in the direction opposite to joint movement.**

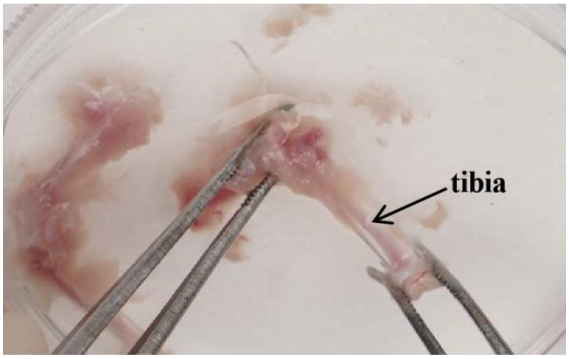


**Figure 3c. Pry it apart in the direction opposite to joint movement.**

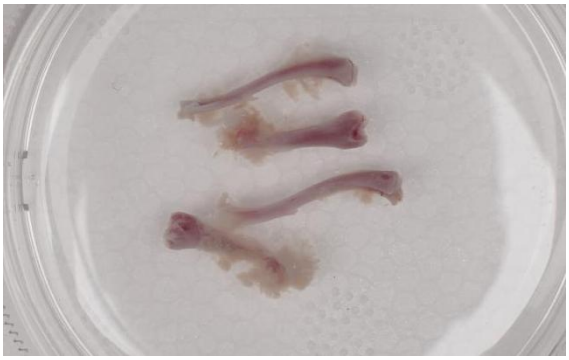

**Figure 4. separate the complete femur and tibia.**

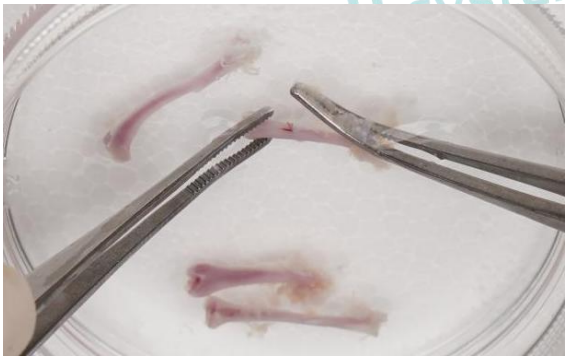

**Figure 5. Remove the residual muscle tissue on the surface of femur and tibia.**

**Figure 6. Pure femur and tibia.**

**Figure 7a.Cut off both ends of the bone.**

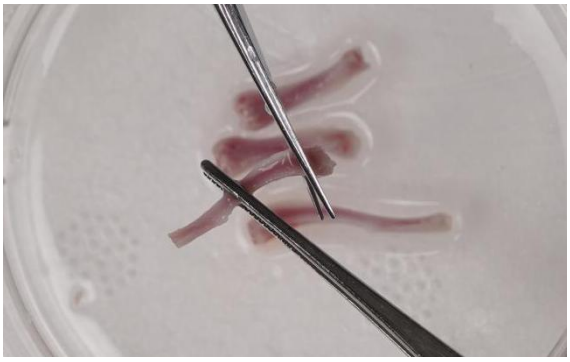

**Figure 7b. Cut off both ends of the bone.**

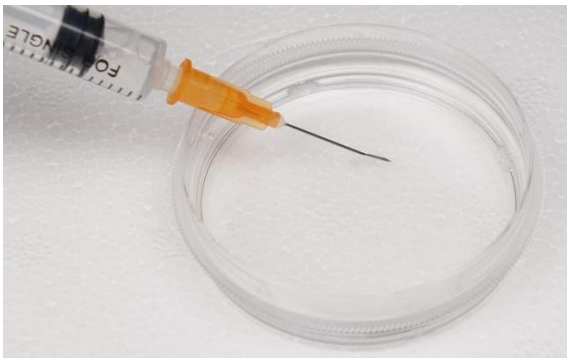

**Figure 8.Draw the Specialized Washing Solution.**

**Figure 9.Stir the bone marrow with the needle and flush it.**

**Figure 10.The bone turns white and translucent.**

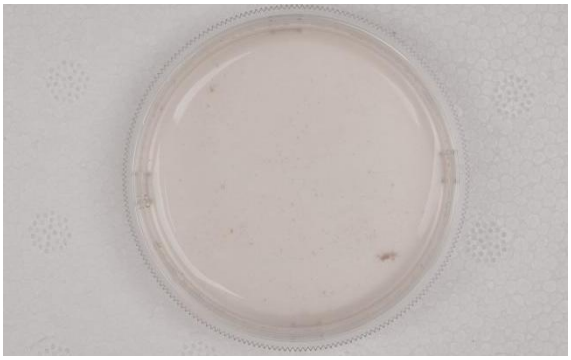

**Figure 11.Collect the bone marrow fluid in the culture dish.**